

Introduction to flaviviral envelope glycoprotein E

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During the last two decades, dengue fever and dengue haemorrhagic fever have emerged globally as a major public health concern [CDC 2008; WHO 2008]. Both conditions are caused by infection with the dengue virus, a flavivirus that is now endemic in more than 100 countries. Both the disease and its vector are prevalent in Africa, the Americas, Australia, India, the Pacific and South-East Asia. Dengue fever is the most common mosquito-borne viral disease for humans, and the vector of transmission is the day-active mosquito *Aedes aegypti*. The life-cycle of the dengue virus is completed in humans, where the outcome can be deadly, especially in children and adolescents [CDC 2008]. Currently there are no anti-viral drugs available that can be used to treat or inhibit infection by the dengue virus [WHO 2008].

The dengue virus belongs to the *Flaviviridae* family, that also contains the viruses causing tick-borne encephalitis (TBE), Japanese encephalitis, yellow fever and West Nile fever. Like all flaviviruses, the dengue virus is organised as an enveloped particle that contains the viral RNA. In all enveloped viruses, the nucleic acid is surrounded by a membrane that is covered entirely by membrane-anchored viral envelope proteins [Schibli & Weissenhorn 2004; Söllner 2004]. As a first step towards infection, the entire viral particle is taken up into an endosome through endocytosis by the host cell [Marsh 1984].

A central event in the invasion of a host cell by an enveloped virus is the fusion of the viral and the cell membrane. The flaviviral envelope consists of 90 dimers of the membrane-anchored envelope glycoprotein E, that cover the surface of the viral membrane [Stiasny &

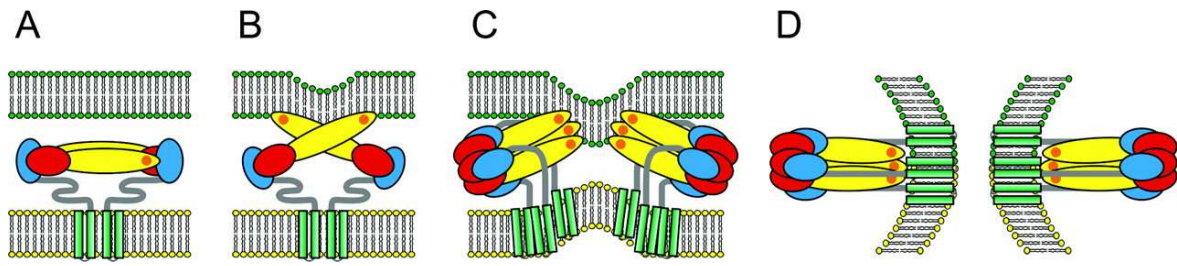


Figure 3.1 Schematic diagram of a model of the flaviviral membrane fusion process, proposed by Heinz *et al.* [Stiasny *et al.* 2005]. A) The E protein homo-dimer in its native state at the surface of the mature virion (lower bilayer). B) Low-pH-induced dissociation of the E dimer and interaction of the fusion peptide loop (orange dot) with the endosomal membrane (upper bilayer). C) Formation of an E trimer (including the flipping back of domain III (blue)) proceeding via still undefined intermediates. D) Formation of the final post-fusion conformation through interactions of the stem-anchor region with domain II (yellow), leading to the juxtaposition of the fusion peptide loops and the membrane anchors (green cylinders) in the fused membrane. Original image by Stiasny *et al.* [2005].

Heinz 2006]. Each identical subunit of the flaviviral E protein contains about 500 residues. As in other low-pH-dependent viruses, the conformation of the envelope protein is pH-dependent [Modis *et al.* 2004; Skehel *et al.* 1982; Zhang *et al.* 2004]. Specifically, the acidification of the endosomal pH is believed to trigger a large-scale conformational change of the E protein [Schibli & Weissenhorn 2004; Zimmerberg *et al.* 1993]. This in turn is associated with the fusion of the viral membrane with the endosomal membrane of the host cell [Gollins & Porterfield 1986; Helenius *et al.* 1980; Stiasny & Heinz 2006], allowing the viral RNA to access and infect the cell through the fusion stalk. Although the exact mechanisms of viral protein-mediated membrane fusion are not known, a number of models have been proposed [Gibbons *et al.* 2003; Harrison 2008; Helenius 1995; Pombourios *et al.* 1999]. Figure 3.1 shows a schematic of one model of flaviviral membrane fusion induced by the low-pH dependent conformational changes of the E protein [Stiasny *et al.* 2005].

The primary differences between the pre- and the post-fusion conformation of the E protein are generally considered to be the irreversible, oligomeric rearrangement from dimers to trimers [Allison *et al.* 1995; Bressanelli *et al.* 2004], and the displacement of domain III relative to domains I and II by 3.3 nm [Bressanelli *et al.* 2004; Modis *et al.* 2003, 2004; Rey *et al.* 1995]. The conformation-specific position of domain III is characterised by associated changes of the domain interfaces; X-ray crystal structures of the pre- and post-fusion conformation are shown in Figure 3.2. One essential aspect of viral fusion is the irreversibility of the structural changes of the fusion protein following exposure to low pH, ultimately resulting in an inactive fusion protein. This suggests that the fusion-active form of the E protein is an unstable intermediate of the low-pH-dependent conformational change. In flaviviruses so-called fusion inactivation, the premature activation of the fusion protein, prevents the

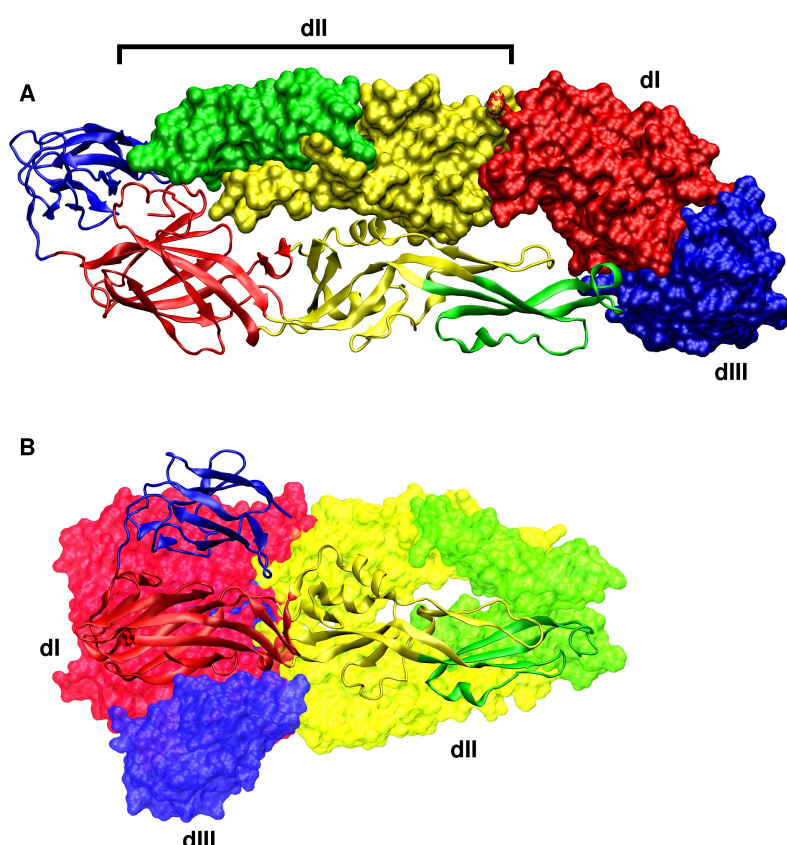


Figure 3.2 X-ray crystal structures of the dengue viral type 2 envelope protein soluble ectodomain sE, A) in the dimeric, pre-fusion conformation, and B) in the trimeric, post-fusion conformation (PDB IDs: 1OAN and 1OK8 respectively). The identical rotamers are divided into three domains, dl (red), dII (yellow/green) and dIII (blue) [Rey *et al.* 1995]. The MMDB database identified the elongated part of domain II in the post-fusion conformation as a fourth homology domain (green) [Chen *et al.* 2003; Wang *et al.* 2007].

infection of the host cell [Heinz 2003; Hurrelbrink & McMinn 2001; Mandl 2005; Mandl *et al.* 2001; McMinn 1997]. Therefore an understanding of the process of the activation of flaviviral envelope glycoproteins, in particular of the factors that trigger the conformational change, would be an important step towards finding ways to manipulate this critical step in the infection process, e.g. in the design of an anti-flaviviral drug.

From X-ray crystallographic and cryo-electron-microscopic structures it is known that the class II viral envelope proteins from flaviviruses and alphaviruses are very similar in the structural organisation of the domains and subunits [Bressanelli *et al.* 2004; Gibbons *et al.* 2004; Kuhn *et al.* 2002; Modis *et al.* 2004; Zhang *et al.* 2003, 2002]. The pH threshold for fusion depends on the specific virus. For various flaviviruses the pH of fusion was determined to be $\text{pH} \approx 6.6$ [Stiasny & Heinz 2006]. In tick-borne encephalitis virus (TBEV) the soluble ectodomain of E was found to be activated by acidic $\text{pH} < 6.5$ [Allison *et al.* 1995]. The fact that the pH of fusion is similar to the pK_a of protonation of histidine in water ($\text{pK}_a = 6.0$) strongly suggests that the process is triggered by the protonation of one or more histidine residues [Kampmann *et al.* 2006]. A number of histidine residues in the E protein dimer are located at the domain and subunit interface, which implies that they play a significant role in the structural stability of the dimer. Figure 3.3 shows the positions of the histidines in the E dimer.

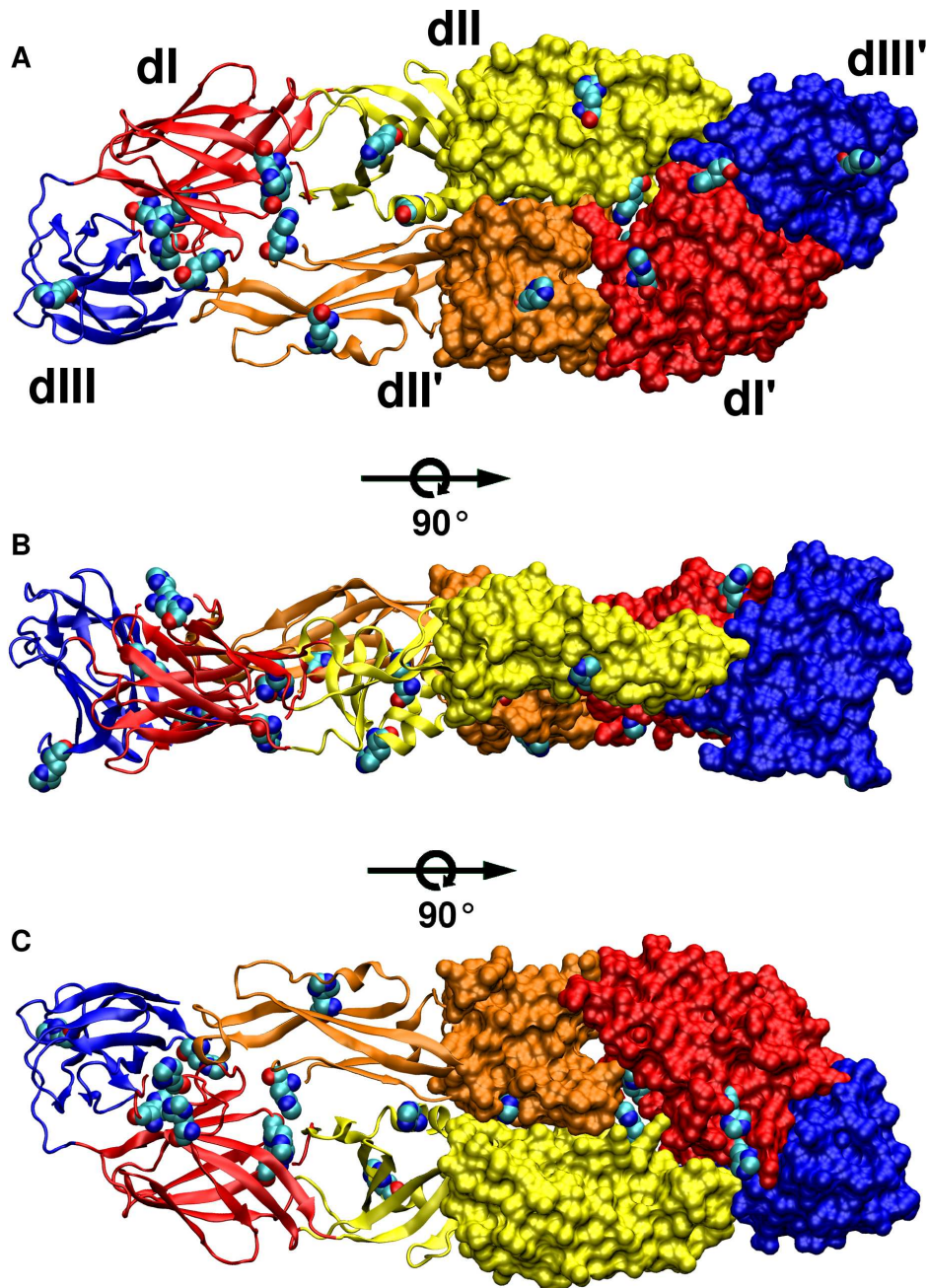


Figure 3.3 The pre-fusion conformation of the mature DEN2 sE protein (10KE [Modis *et al.* 2003]) and the positions of the histidines in the dimer. The histidine residues are represented as spheres. A) View onto the inner envelope surface (*in situ* facing the viral membrane), B) rotated by 90° and C) top view onto the outer envelope surface. The solvent-accessible surface was rendered for one half of the structure, the other half is shown in cartoon representation. Colour legend according to domains: red – domains dl; yellow and orange – dll of the two subunits respectively; blue – dlll; the domains of one subunit of the dimer are labelled dl-III, of the other subunit dl'-dlll'.

The structural similarity and the low-pH dependency of the envelope proteins throughout the *Flavivirus* family and in other virus families strongly suggest a common fusion activation mechanism. The following chapters of this thesis present the details of this hypothesis, and the molecular modelling studies that were undertaken to test it. Chapter 4 introduces the histidine-switch hypothesis and reviews the different modelling approaches undertaken to test this hypothesis. Chapter 5 presents the molecular modelling and MD simulation study of the dengue viral type 2 envelope glycoprotein soluble ectodomain sE undertaken to investigate the effect of histidine protonation on a pH-dependent fusion protein. Chapter 6 introduces a model for the fusion activation mechanism of flaviviral envelope proteins that is based on the results of the MD simulation study.
